IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Tuszynski, Mark H.

Title: METHODS FOR THERAPY OF NEURODEGENERATIVE DISEASE OF THE BRAIN

Appl. No.: 10/032,952

Filing Date: 10/26/2001

Examiner: Chen, Shin Lin

Art Unit: 1632

CERTIFICATE OF EXPRESS MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service's "Express Mail Post Office To Addressee" service under 37 C.F.R. § 1.10 on the date indicated below and is addressed to: Commissioner for Patents, PO Box 1450, Alexandria, Virginia 22313-1450.

EL990322346US (Express Mail Label Number)
December 31, 2003 (Date of Deposit)

Michelle Symmson (Printed Name)

Michelle Symmson (Date of Deposit)

DECLARATION OF DR. MARK H. TUSZYNSKI UNDER 37 CFR 1.132

Mail Stop NON-FEE AMENDMENT
Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

I, Dr. Mark H. Tuszynski, declare as follows:

1. I am a Professor of Neurosciences and Director of the Center for Neural Repair at the University of California, San Diego (UCSD). I practice medicine as an attending neurologist at the UCSD and VA Medical Centers in La Jolla, California, and as the lead neurologist in ongoing clinical trials for treatment of Alzheimer's Disease (AD) using gene therapy in humans. I am also an inventor of the invention claimed in this US Patent Application Serial No. 10/032952 (methods for *in vivo* gene therapy of neurodegenerative conditions), and in U.S. Patent No. 6,167,888 (claiming methods for *ex vivo* gene therapy of neurodegenerative conditions).

2. Age-related changes in cortical cholinergic innervation in diverse cortical regions were substantially reversed by cellular gene delivery of NGF. For example, aged monkeys that received grafts of NGF-secreting cells exhibited a substantial and significant reversal of age-related declines in cortical cholinergic innervation (Figs. 3B and 3D).

When averaged across all cortical regions examined, NGF-grafted animals had levels of cholinergic innervation that were significantly greater than values of aged control monkeys ($p < 0.0001$, post-hoc Fischer's) and were equal to intact young monkeys ($p = 0.89$, post-hoc Fischer's).

3. Further, cortical regions (insular and inferior temporal cortices) receiving innervation primarily from the intermediate division of Ch4, the cholinergic subdivision targeted for grafting, demonstrated levels of cholinergic innervation significantly ***exceeding*** those of normal young monkeys (overall 13.4 ± 4.5% increase relative to young monkeys; $p = 0.01$, post-hoc Fischer's; Fig. 3A and 3D). Levels of cholinergic innervation in these regions also significantly exceeded control-aged monkeys (overall 43.6 ± 3.0% increase; $p < 0.0001$, post-hoc Fischer's). Cholinergic fiber densities in cortical regions (cingulate and frontal cortex, hippocampus) not heavily innervated by the targeted Ch4i cell population also exhibited reversal of age-related losses after NGF cell grafting, although the magnitude of the reversal (20.6 ± 4.1% increase; $p = 0.01$, post-hoc Fischer's) was more modest than that observed in temporal and insular cortex.

4. These effects of cellularly-delivered NGF on cortical cholinergic innervation were exerted at a distance, since the growth factor was presented to the cholinergic soma yet influenced terminal axon density in the distant cortex of the primate brain.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

_____ *Signed copy to be submitted*

Dated Dr. Mark H. Tuszynski